Region-specific roles of the corticotropin-releasing factor–urocortin system in stress

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Abstract | Dysregulation of the corticotropin-releasing factor (CRF)–urocortin (UCN) system has been implicated in stress-related psychopathologies such as depression and anxiety. It has been proposed that CRF–CRF receptor type 1 (CRFR1) signalling promotes the stress response and anxiety-like behaviour, whereas UCNs and CRFR2 activation mediate stress recovery and the restoration of homeostasis. Recent findings, however, provide clear evidence that this view is overly simplistic. Instead, a more complex picture has emerged that suggests that there are brain region- and cell type-specific effects of CRFR signalling that are influenced by the individual's prior experience and that shape molecular, cellular and ultimately behavioural responses to stressful challenges.

Controllable stress
A stress paradigm in which exposure to a stressor (usually footshock or tailshock) can either be avoided or escaped.

Learned helplessness
A paradigm in which exposure to a severe inescapable stressor induces 'helpless' behaviour when it becomes possible to escape the stressor.

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Since its initial identification and characterization[1], corticotropin-releasing factor (CRF) has been shown to have a major role in coordinating the endocrine, autonomic and behavioural response to stress[2,3]. CRF is released into the periphery from the paraventricular nucleus of the hypothalamus (PVN) and steers the activation of the hypothalamic–pituitary–adrenal (HPA) axis by triggering the release of adrenocorticotropic hormone from the anterior pituitary, which in turn stimulates the synthesis and secretion of glucocorticoids from the adrenal gland[1]. CRF also steers the sympathetic stress response by acting on the locus coeruleus (LC), adrenal medulla and peripheral sympathetic nervous system[4,5]. CRF plays a crucial part in coordinating the peripheral stress-response systems and the central release of noradrenaline in reaction to stressful challenges.

However, centrally released CRF also contributes directly to (and may even be necessary for) a behavioural state of anxiety, independently of its effects on the pituitary and sympathetic systems[3,6]. In rodents, central overexpression of CRF induces an anxiogenic behavioural phenotype[2,7,8], whereas suppressing CRF expression has anxiolytic effects in both basal anxiety[9] and stress-induced anxiety[10]. Interestingly, CRF levels are elevated in the CNS of individuals affected by stress-related psychiatric diseases such as major depressive disorder[11] and post-traumatic stress disorder (PTSD)[12], and in some cases are normalized after antidepressant treatment[13,14].

The anxiogenic effects of CRF have been attributed to the activation of CRF receptor type 1 (CRFR1), one of the two G protein-coupled receptors to which CRF binds (BOX 1). CRFR1 blockade in rodents prevented the CRF-induced anxiogenic phenotype[15], and mice lacking CRFR1 display reduced anxiety-like behaviour as assessed in a wide range of anxiety-related tests[16–18]. These findings have led to a suggested (causative) role for CRFR1 overactivation in stress-related psychopathologies, triggering the development of CRFR1 antagonists as potential next-generation anxiolytics and antidepressants, with varying levels of success (BOX 2).

In contrast to CRFR1, the role of CRFR2 activation in mediating anxiety and depression has been less clear, and two prominent theories circulate to explain its role. The most common view is that CRFR2 activation is responsible for ensuring physiological and psychological homeostasis and counteracts the initial stress-response-provoking effects and anxiety-like behaviours induced by CRFR1 activation[3]. This view is primarily based on evidence obtained from *Crfr2*-knockout mice, which display an increased corticosterone response to stress[19,20], an anxiogenic phenotype[19,21] and impaired stress recovery[22] (the latter of which was also seen in mice lacking all three urocortins (UCN1–UCN3), the primary ligands for CRFR2 (REF. 23) (BOX 1). The alternative hypothesis of the role of CRFR2 is that CRFR1 and CRFR2 contribute to opposite modes of stress-coping behaviour, with CRFR1 mediating active defensive behaviour (as triggered by controllable stress) and CRFR2 mediating passive coping behaviour and depression-like responses (such as learned helplessness, which is triggered by uncontrollable stress)[24,25]. This hypothesis is primarily based on the observation that intact CRFR2 signalling in the dorsal raphe nucleus

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Uncontrollable stress
A paradigm in which exposure to a stressor (usually footshock or tailshock) is unavoidable and inescapable.

(dorsal RN) is required for the sensitization of serotonergic neurons and development of a depressive-like phenotype resulting from inescapable stress[24].

However, many reported findings seem to contradict these circulating views on CRFR signalling[26–28]. These discrepant findings could potentially be attributed to an improper delineation of the contribution of CRF-related peptides and their receptors to the observed effects, as a consequence of their partially overlapping distribution patterns (FIG. 1). Pharmacological studies have often lacked absolute specificity of receptor activation; high ligand concentrations act on both receptor types, and receptor antagonists lack true specificity[29]. In addition, studies involving mice that constitutively lack the ligands or receptors are complicated by possible compensatory developmental changes and pleiotropic effects, including disturbances of the HPA axis that may result from global gene inactivation. The emergence of new neurobiological tools, such as conditional mutagenesis, viral manipulations and optogenetics, has allowed site-specific manipulation of CRFR1 and CRFR2 signalling. However, it has also provided data that challenge the somewhat simplistic view that CRF–CRFR1 signalling induces anxiety and UCN–CRFR2 signalling promotes stress recovery to

Box 1 | The CRF–UCN system

The family of corticotropin-releasing factor (CRF)-related peptides includes CRF, urocortin 1 (UCN1), UCN2 (also known as stresscopin-related peptide), UCN3 (also known as stresscopin) and two non-mammalian peptides — the fish Urotensin-1 and the amphibian sauvagine[183]. These 38–41-amino-acid peptides are structurally related and share relatively high (26–54%) sequence identity[183].

They act by binding to two different G protein-coupled receptors: CRF receptor 1 (CRFR1) and CRFR2. These receptors are encoded by distinct genes and have numerous splice variants (including CRFR1α and CRFR1β, and CRFR2α (in the figure, with amino terminus indicated by 'α'), CRFR2β (in the figure, with N terminus indicated by 'β') and CRFR2γ), several of which are non-functional[175,184], and these variants are expressed in various central and peripheral tissues[185–187]. The CRFRs exhibit ~70% sequence homology, with most variation found in the ligand-binding domain. CRF is a high-affinity ligand for CRFR1, UCN1 binds with equal affinity to both receptors[188], and UCN2 and UCN3 are the preferred ligands for CRFR2 (REFS 171,173) (see figure; numbers next to arrows indicate half-maximal inhibitory concentration (IC_{50}) values). However, specificity may be concentration dependent, as high concentrations of CRF also activate CRFR2 (REF. 188).

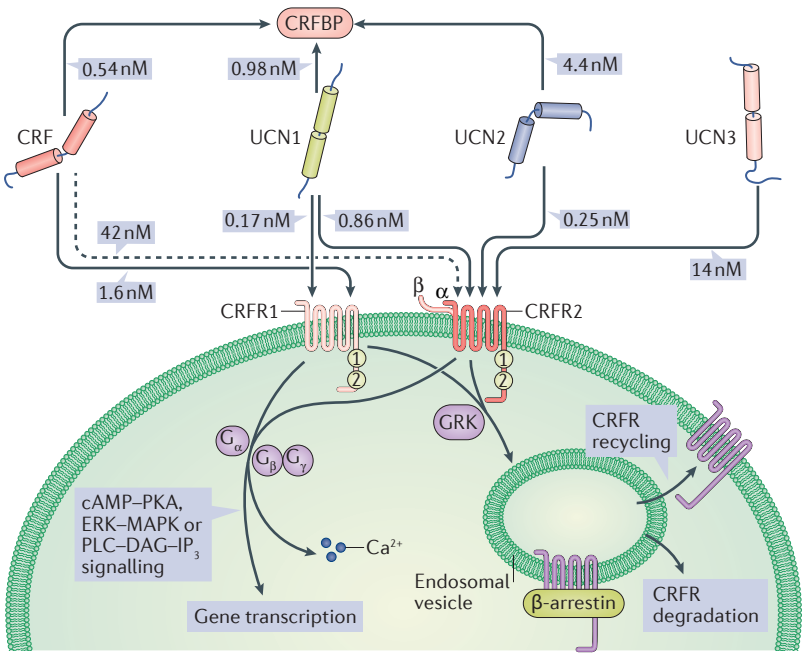
The action of the ligands is modulated by binding to CRF-binding protein (CRFBP), which limits ligand availability and serves as a local reservoir[189]. However, CRFBP may have a broader role in modulating the effects of CRFR–ligand binding[190] that deserves further investigation. In the ventral tegmental area (VTA), for example, the effects of CRFR2 activation crucially depend on CRFBP[98,151], and recent *in vitro* evidence suggests a role for CRFBP in escorting CRFR2 to the cell surface[191].

Activated CRFR1 and CRFR2 primarily signal by G_s protein coupling, resulting in the induction of the cyclic AMP–protein kinase A (PKA) and the extracellular signal-regulated kinase–mitogen-activated protein kinase (ERK–MAPK) pathways. These signals induce the transcription of downstream target genes[192], thus regulating synaptic plasticity processes such as dendrite stabilization, ion channel transmission, transcription of cAMP-responsive element-binding protein (*CREB*) and other genes, and receptor scaffolding, trafficking and crosstalk. G_s-coupled processes also lead to intracellular Ca^{2+} mobilization[193]. CRFRs also interact with other G-protein systems, including $G_q\alpha$, G_i, G_o, $G_{i1/2}$ and G_z, thus activating phospholipase C variants (PLCs) and resulting in the activation of ERK1 and ERK2 and an increase in intracellular Ca^{2+} concentration[174]. Thus, depending on their localization and cellular context, CRFRs can have many different effects, in a concentration-dependent manner[175].

After CRFR binding, G protein-coupled receptor kinases (GRKs) rapidly phosphorylate the receptors, desensitizing them and increasing their affinity for β-arrestins, and then translocate to the cell surface to uncouple the CRFR from the G protein and 'arrest' signal transduction. These processes can also alter receptor signalling pathways. Moreover, β-arrestins enable the internalization of the desensitized CRFR1 (REF. 193) and CRFR2 (REF. 178), which are then either dephosphorylated, resensitized and returned to the plasma membrane, or — with long-term exposure to high agonist concentrations — degraded in lysosomes, resulting in a decrease in the number of CRFRs[194–196]. Severe stress exposure seems to modulate these processes and promote the degradation of CRFRs[34]. Importantly, not all phosphorylated receptors are internalized; some remain at the membrane[197]. This might be particularly important during recurrent exposure to stress, and failure of this regulation may result in severe anxiety and possibly depression.

Besides phosphorylation (1), CRFR activity is modulated by carboxy-terminal PDZ-domain interactions (2). PDZ-domain binding by membrane-associated guanylate kinases (MAGUKs) influences receptor localization in the cell[198] and may anchor CRFRs to larger signalling complexes[198]. PDZ-domain interactions may modulate, for example, downstream kinase phosphorylation[199], functional crosstalk between receptors[200] and CRFR trafficking[201]. Many other regulatory systems besides these two main regulatory sites of CRFRs seem to modulate CRFR activity but are not well understood and warrant further study.

DAG, diacylglycerol; IP3, inositol-1,4,5-trisphosphate.



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Prepulse inhibition
The neurological phenomenon in which a weaker prestimulus (prepulse) inhibits the response to a subsequent, strong startling stimulus (pulse). Prepulse inhibition is thought to represent sensorimotor gating.

Fatty acid amide hydrolase
A serine hydrolase that is the principal catabolic enzyme for the fatty acid amides (including the endocannabinoid anandamide).

maintain homeostasis. Below, we review recent studies involving brain region- and cell type-specific manipulation of CRFR signalling to improve our understanding of the role of the CRF–UCN system in mediating anxiety- and stress-related behaviours.

Region-specific functions of CRFR1

Owing to the lack of CRFR subtype-specific antibodies, the determination of the patterns of distribution of these receptors in the brain largely relies on *in situ* hybridization histochemistry-based studies (and recently developed reporter mice)[30,31]. Besides the high expression in the anterior pituitary, *Crfr1* mRNA is abundantly expressed throughout the brain, with high levels in the cerebellum and neocortical, limbic, midbrain and brainstem regions, moderate levels in the dorsal and median RN, and only low levels in the PVN[32,33] (FIG. 1b). Identification of the neuronal cell types (for example, glutamatergic or serotonergic neurons) in which *Crfr1* mRNA is expressed has been complicated by its low expression levels, which challenge its detection, and by an inability to resolve its exact site of action in these cells (that is, presynaptic or postsynaptic).

Studies using electrophysiological manipulations (TABLE 1) and reporter mice[30,31] have considerably improved our insight into the sites of action of CRFR1 (FIG. 2a), but a large terrain still remains to be explored. For example, CRFR1 function is highly dependent on its subcellular distribution, which is tightly regulated by its internalization and subsequent degradation or recycling[34] (BOX 1). Electron microscopy-based studies provide important information on these processes and their regulation by stress exposure[35]. To shed light on the complex and temporally and spatially fine-tuned actions of CRFR1, here we review studies of CRFR1 signalling in sites that are involved in mediating anxiety-like behaviour: the extended amygdala, hippocampus, medial prefrontal cortex (mPFC), ventral tegmental area (VTA) and brainstem regions including the LC, RN and periaqueductal grey (PAG) (FIG. 2a; TABLE 1).

Extended amygdala. The extended amygdala is involved in the acquisition and expression of fear and anxiety[36], processes that seem to be boosted by local CRFR1 signalling. For example, in the basolateral amygdala (BLA) — a region important for fear learning and fear-memory consolidation[37] — CRF–CRFR1 signalling dose-dependently activates glutamatergic neurons[38]. *In vitro* electrophysiology-based studies revealed that activation of BLA CRFR1 increases neuronal excitability[39] and induces long-term potentiation (LTP)[40], whereas selective deletion of *Crfr1* from glutamatergic neurons in the BLA decreases glutamatergic neurotransmission[40].

Injection of CRF into the BLA dose-dependently increases basal anxiety-like behaviour through CRFR1, causing anxiety-like responses during social interaction[41–43] as well as enhanced acquisition of fear-potentiated startle and impaired prepulse inhibition[44]. CRF in the BLA impairs the consolidation of fear extinction[45] but improves consolidation of the fear memory itself[46]. CRF achieves the latter effect by interacting with the β-adrenoceptor–cyclic AMP cascade, thus facilitating the memory-modulating effects of noradrenergic stimulation in this region[47] — an effect that can be blocked by local CRFR1 antagonism[48].

BLA-specific RNAi-mediated knockdown of *Crfr1* decreases anxiety levels in tests that target rodents' aversion to open spaces, and mimics the anxiolytic effects of environmental enrichment, which itself is associated with very low BLA levels of *Crfr1* mRNA[49], suggesting that CRFR1 signalling mediates the anxiogenic effect of CRF in this region. These anxiogenic effects of CRF–CRFR1 signalling seem to be established through interaction with the local endocannabinoid system; they can be prevented by locally blocking the CRFR1-mediated induction of the enzyme fatty acid amide hydrolase and thus decreasing levels of its substrate, the endocannabinoid anandamide[50].

A more complex role for CRFR1 signalling arises in the central amygdala (CeA). This region serves as the major output nucleus of the amygdala, as it connects to

Box 2 | Treating stress-related mental disorders with CRFR1 antagonists

Compelling evidence implicates a dysfunctional corticotropin-releasing factor (CRF) system in stress-related mental disorders such as major depressive disorder and post-traumatic stress disorder[168]. This has caused a surge in the development of anxiolytics and antidepressants targeting the CRF system, with varying success[202]. The first clinical studies performed with two CRF receptor 1 (CRFR1) antagonists developed by Neurocrine Biosciences, NBI-30775 (also known as R121919; phase IIa) and NBI-34041 (also known as SB 723620; phase I), indicated dose-dependent improvements in patient- and clinician-rated depression and anxiety scores in people with depression. Moreover, although responsiveness of the hypothalamic–pituitary–adrenal axis to intravenous CRF was not affected, its hormonal response to a psychosocial stressor (which is often increased in depression[203]) was reduced by both of these compounds[204,205], thus indicating their clinical relevance. Moreover, administration of NBI-30775 seemed to normalize the sleep disturbances that are often observed in people with depression, by increasing the ratio of slow-wave sleep to phasic REM (rapid eye movement) sleep[206]. However, other studies indicated no increase in efficacy of such agents versus traditional serotonin-specific reuptake inhibitors (SSRIs) in major depressive disorder[207], and reliable biomarkers would be needed to identify patients who would particularly benefit from CRFR1 antagonists.

One such biomarker could be the sleep electroencephalogram (EEG), as patients with depression display decreased slow-wave sleep and disinhibited REM sleep[187]. These sleep disturbances were mimicked in a CRF-overexpressing mouse line[208] and were blocked in patients by CRFR1 antagonists[206], implicating CRFR1 in this phenomenon. In patients, the extent of REM sleep disinhibition even predicted the clinical improvement conferred by CRFR1-antagonist treatment[206], further supporting the idea that sleep EEG might be a valuable biomarker for identifying potential responders to CRFR1 antagonists[209]. Other biomarkers may include genetic variations that are indicative of excessive CRF signalling or *CRFR1* polymorphisms that may identify patients in whom disproportionate CRF–CRFR1 signalling is one of the main pathogenic factors.

Notably, when addressing the potential of CRFR1 antagonists as antidepressants, the rapid desensitization and internalization of CRFR1 that are typically observed in the presence of high ligand concentrations (BOX 1) make it unlikely that CRF hypersecretion alone is sufficient to account for the enhancement of central CRF neurotransmission in depression, and limits the effectiveness of CRFR1 antagonists as anxiolytic drugs. Considering the much higher frequency of depression in women[210], there may be important sex differences in the mechanisms regulating receptor internalization and its response to stress; for example, women show less CRFR1 internalization, owing to reduced binding to β-arrestin 2 (REFS 211,212). Therefore, genetic or acquired abnormalities in the function of G protein-coupled receptor kinases (GRKs) and β-arrestins may also be involved in the pathophysiology of stress-related anxiety and depression and should be considered when designing new drug treatments.

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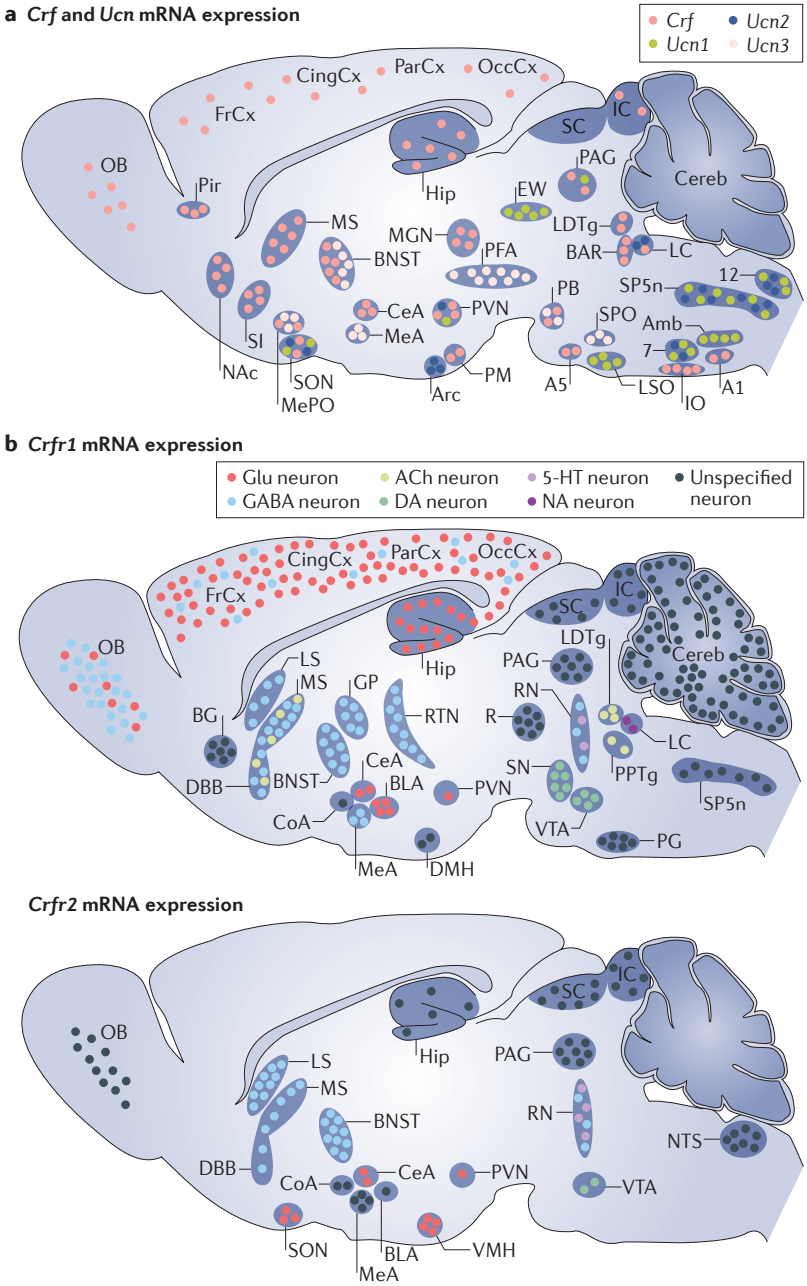

Figure 1 | **mRNA expression of components of the CRF–UCN system. a** | Schematic representation of corticotropin-releasing factor (*Crf*), urocortin 1 (*Ucn1*), *Ucn2* and *Ucn3* mRNA distribution in a sagittal section of the rodent brain. Although *Crf* mRNA is expressed widely throughout the brain, mRNA expression of *Ucn* mRNA is much more restricted. The image is based on data from REFS 170–173. **b** | Schematic representation of CRF receptor 1 (*Crfr1*) mRNA distribution in different neuronal cell types (glutamatergic, GABAergic, cholinergic, serotonergic, dopaminergic, noradrenergic or unspecified) in a sagittal section of the rodent brain (top panel). Schematic representation of *Crfr2* mRNA distribution in different neuronal cell types in a sagittal section of the rodent brain (bottom panel). *Crfr1* mRNA is widely distributed throughout the brain, whereas *Crfr2* mRNA has a much more localized distribution pattern. Images are based on data from REFS 30–33,40,145,151, 152,169,222. 5-HT, 5-hydroxytryptamine (serotonin); 7, facial nucleus; 12, hypoglossal nucleus; A1, A1 noradrenaline cells; A5, A5 noradrenaline cells; ACh, acetylcholine; Amb, ambiguous nucleus; Arc, arcuate nucleus; BAR, Barrington's nucleus; BG, basal ganglia; BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CeA, central amygdala; Cereb, cerebellum; CingCx, cingulate cortex; CoA, cortical amygdala; DA, dopamine; DBB, diagonal band of Broca; DMH, dorsomedial hypothalamus; EW, Edinger–Westphal nucleus; FrCx, frontal cortex; Glu, glutamate; GP, globus pallidus; Hip, hippocampus; IC, inferior colliculus; IO, inferior olive; LC, locus coeruleus; LDTg, laterodorsal tegmental nucleus; LS, lateral septum; LSO, lateral superior olive; MeA, medial amygdala; MePO, medial preoptic area; MGN, medial geniculate nucleus; MS, medial septum; NA, noradrenaline; NAc, nucleus accumbens; NTS, nucleus of the solitary tract; OB, olfactory bulb; OccCx, occipital cortex; PAG, periaqueductal grey; ParCx, parietal cortex; PB, parabrachial nucleus; PFA, perifornical area; PG, pontine grey; Pir, piriform cortex; PM, premammillary nucleus of the hypothalamus; PPTg, pedunculopontine tegmental nucleus; PVN, paraventricular nucleus of the hypothalamus; R, red nucleus; RN, raphe nucleus; RTN, reticular nucleus; SC, superior colliculus; SI, substantia innominata; SN, substantia nigra; SON, supraoptic nucleus; SP5n, spinal trigeminus nucleus; SPO, superior paraolivary nucleus; VMH, ventromedial hypothalamus; VTA, ventral tegmental area.

brainstem areas[51] and the lateral hypothalamic area[52], and thus controls the expression of innate behaviours and physiological responses. Owing to the high levels of CRF in this subnucleus, many electrophysiological and behavioural studies have investigated the effects of local CRF release, although initially without strong emphasis on the exact CRFR subtype mediating the effects. Many studies have shown that CRF affects both GABAergic and glutamatergic signalling in the CeA (reviewed in REF. 53), but there have been many apparently contradictory reports of the effects of CRF on CeA neurons, with described effects ranging from excitatory[54–57] to inhibitory[39,58–60].

Presynaptic CRFR1 on GABAergic neurons in the CeA has been consistently shown to increase local GABA release — a response that seems to promote anxiety[61]. By contrast, postsynaptic CRFR1 on glutamatergic neurons in this region has been reported to depress glutamatergic transmission[58]. However, other studies showed that presynaptic CRFR1 in the CeA contributes to the increase in spontaneous glutamate release induced by CRF[55], facilitates synaptic transmission evoked by mechanical stimulation (of the knee) by activating protein kinase A (PKA)[56] and increases excitability[57]. These inconsistencies could potentially be caused by a highly flexible responding (that is, plasticity) of the CRF system to different background levels of stress in the CeA, with CRF having predominantly inhibitory effects at low-stress levels but boosting glutamatergic transmission and inducing LTP against a background of stress (induced by, for example, daily handling, daily injections, chronic pain or drug withdrawal).

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Table 1 | **Neuronal effects of regional manipulation of CRFR1-mediated signalling**

Brain region	CRFR1 manipulation*	Neuronal effects	Role for CRFR1 signalling	Refs
Amygdala				
Basolateral amygdala	Activation	Reduced slow AHP, increased firing frequency	Excitation	39
		Increased evoked fEPSPs (LTP)	Excitation	40
		Activation of glutamatergic neurons (FOS expression)	Excitation	38
	Inhibition	Blockage of CRF-induced LTP	Excitation	40
Central amygdala	Activation, low stress	Hyperpolarization of the resting membrane potential, decreased membrane input resistance, reduced slow AHP, no effect on overall firing frequency	–	39
		Reduced mEPSC amplitude, depressed EPSCs	Inhibition	58
		Enhanced IPSC amplitude	Inhibition	59,60
		Increased mIPSC frequency	Inhibition	60
	Activation, high stress	Increased whole-cell Ca^{2+} current	Excitation	54
		Increased sEPSC frequency	Excitation	55
		Increased LTP	Excitation	223
	Inhibition, low stress	Prevented CRF-potentiated evoked EPSCs	Excitation	56
		No effect on evoked EPSCs	–	57
	Inhibition, high stress	Potentiated evoked EPSCs	Inhibition	58
		Inhibited potentiation of evoked EPSCs by pain	Excitation	57
BNST	Activation	Increased sEPSC frequency	Excitation	71,72,74
		Increased evoked IPSCs (enhanced postsynaptic responding to GABA) in vlBNST	Inhibition	224
	Inhibition	Reduced sEPSC frequency	Excitation	71
		Prevented increase in sEPSC frequency during drug withdrawal	Excitation	72
Midbrain				
Ventral tegmental area	Activation, low stress	No effect on glutamate release	–	95
		Temporary minimal increase of NMDAR currents	Excitation	96,97
		No effect on AMPAR currents	–	97
		Increased firing rate of GABAergic and DA neurons	Excitation	91
		Potentiated evoked EPSCs	Excitation	92
		Increased DA neuron firing	Excitation	93
		Increased local glutamate release‡	Excitation	95
		Prolonged and potentiated NMDAR currents‡	Excitation	96
	Activation, high stress	Potentiated AMPAR currents, increased AMPAR mEPSC frequency‡	Excitation	97
	Inhibition, low stress	Increased DA neuron population activity	Inhibition	225
	Chronic inactivation in DA cells‖	Reduced stress-induced DA release in the prefrontal cortex§	Excitation	40
Brainstem				
Locus coeruleus	Activation	Increased firing rate	Excitation	117
		Increased neuronal activity (FOS)	Excitation	110
	Inhibition	Reduced CRF-induced potentiated firing	Excitation	117
Raphe nuclei	Activation	Inhibited neuronal firing	Inhibition	128
		Reduced serotonin release in the nucleus accumbens, lateral striatum and lateral septum	Inhibition	129–131

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Table 1 (cont.) | **Neuronal effects of regional manipulation of CRFR1-mediated signalling**

Brain region	CRFR1 manipulation*	Neuronal effects	Role for CRFR1 signalling	Refs
Other				
Hippocampus	Activation, low stress	Increased excitability	Excitation	79
		Potentiated synaptic plasticity and efficacy	Excitation	226
		Primed and augmented LTP	Excitation	40,80
	Prolonged activation	Reduced fEPSPs, impaired STP	Inhibition	215
	Chronic inhibition	Prevented depressed synaptic transmission and impaired synaptic plasticity (reduced fEPSPs and STP) caused by early-life stress§	Inhibition	227
Medial prefrontal cortex	Activation	No net effect, but strong NMDAR-mediated depression of fEPSPs in partly disinhibited slices	–	228

AHP, afterhyperpolarization; AMPAR, AMPA receptor; BNST, bed nucleus of the stria terminalis; CRF, corticotropin-releasing factor; CRFR1, CRF receptor 1; DA, dopamine; EPSC, excitatory postsynaptic current; fEPSP, field excitatory postsynaptic potential; IPSC, inhibitory postsynaptic current; LTP, long-term potentiation; mEPSC, miniature EPSC; mIPSC, miniature IPSC; NMDAR, NMDA receptor; sEPSC; spontaneous EPSC; STP, short-term potentiation; vlBNST, ventrolateral BNST. *Studies describing the effects of acute activation or inhibition of CRFR1 by a single administration of agonist or antagonist, unless specified otherwise. ‡Effect of CRFR1 signalling after repeated administration of drugs of abuse. §Chronic CRFR1 activation or inhibition in transgenic mice with overexpression or knockout of the receptor, respectively. ‖Manipulation also involved chronic knockdown of CRFR1 expression in dopaminergic cells of the substantia nigra.

These apparent inconsistencies could also be attributed to differences between CeA subregions (for example, lateral versus medial CeA)[53] or to the heterogeneous cell population in the CeA, as extremely divergent effects of CRF on transmission have been observed even within a single study[62]. Future studies aimed at classifying neuronal populations should target these differences.

Behaviourally, CeA CRFR1 blockade does not affect basal anxiety but reduces the anxiogenic effects of immobilization stress[63]. Furthermore, CeA-specific knockdown of *Crfr1* reduced anxiety-related behaviour in animals in the social defeat model[64], and CeA CRFR1 blockade prevented CRF-induced augmentation of pain responses[65] and of pain-related anxiety[66]. A reduced sensitivity of CRFR1-expressing CeA neurons to CRF has been related to anxiolytic effects following stress exposure and has been suggested to reflect stress coping[67]. Although not much is known about the downstream mediators of these effects in the CeA, locally upregulated activity of tissue plasminogen activator (tPA)[68], which has previously been shown to promote stress-induced synaptic plasticity and anxiety-like behaviour[69], has been implicated. Altogether, these findings seem to link CeA CRFR1 activation to an overall anxiogenic response but primarily suggest a complex, plastic role of CRFR1 signalling in the CeA, depending on the exact cell type, current stress level and history of the animal or cell.

The bed nucleus of the stria terminalis (BNST) is involved in sustained states of anxiety[70] and in stress–reward interactions[55]. CRFR1 signalling in this region increases glutamatergic transmission[71,72] and induces anxiety-like behaviour[73]. Moreover, CRFR1 signalling has been shown to be crucial for the modulatory actions of dopamine[71] and noradrenaline[74] on local glutamatergic signalling. BNST CRFR1 inhibition reduces chronic-stress-induced anxiety, hyperalgesia and activation of the HPA axis[75], whereas BNST CRFR1 activation increases stress-induced reinstatement of drug seeking and withdrawal-induced anxiety[76]. Indeed, CRFR1-mediated enhancement of glutamatergic transmission has been proposed to be a possible common effect of multiple substances of abuse[71].

Hippocampus. CRFR1 is present in the hippocampus and may therefore be involved in the stress-mediated modulation of learning and memory. Indeed, CRF in the hippocampus exerts dose- and time-dependent effects[77]: physiological levels of CRF augment hippocampal function acutely, but, over longer timescales, severe stress — which is associated with prolonged exposure to high levels of CRF — impairs hippocampal function by inducing spine retraction and loss of synapses[78]. Although we acknowledge the relevance of the latter effects (BOX 3), we primarily focus on the immediate effects of CRF-induced signalling in the hippocampus. Acute exposure to hippocampal CRF has been shown to reduce neuronal afterhyperpolarization and thus increase neuronal excitability[79], to induce long-lasting potentiation of synaptic plasticity and efficacy, and to facilitate LTP[80].

Behaviourally, hippocampal function is 'potentiated' in response to local CRF signalling; fear learning[80] and retention of fear memory[81] are both improved after hippocampal infusion of CRF, which induces an increase in local expression of brain-derived neurotrophic factor (BDNF) that might potentially mediate these effects[82]. Moreover, hippocampal infusion of CRF was found to increase defensive behaviours and anxiety during unconditioned and conditioned threat exposures and to contribute to the consolidation of conditioned defensive behaviour[83]. These effects are attributed to local CRFR1 activation, as CRF-enhanced fear learning and consolidation were unaffected by the administration of a selective CRFR2 antagonist[28]. The observations that mice lacking *Crfr1* exhibit memory deficits[84], whereas *Crfr2*-knockout mice do not[85], also implicate CRFR1 in these effects. CRF–CRFR1 signalling was recently shown to enhance the propagation of glutamatergic neuronal activity from the dentate gyrus to CA1 (REF. 40). Downstream, hippocampal CRFR1 signalling

Immobilization stress
A stress paradigm in which the animal is (sometimes repeatedly) restrained in a confined space for a certain amount of time, during which it is unable to move.

Social defeat
A phenomenon that typically manifests in a 'resident–intruder' paradigm: the animal (the intruder) is repeatedly placed in the cage of a dominant animal (the resident) in a manner that allows a non-lethal conflict.

Afterhyperpolarization
The hyperpolarizing phase of an action potential during which the cell membrane potential temporarily falls below the normal resting potential by an excessive potassium efflux.

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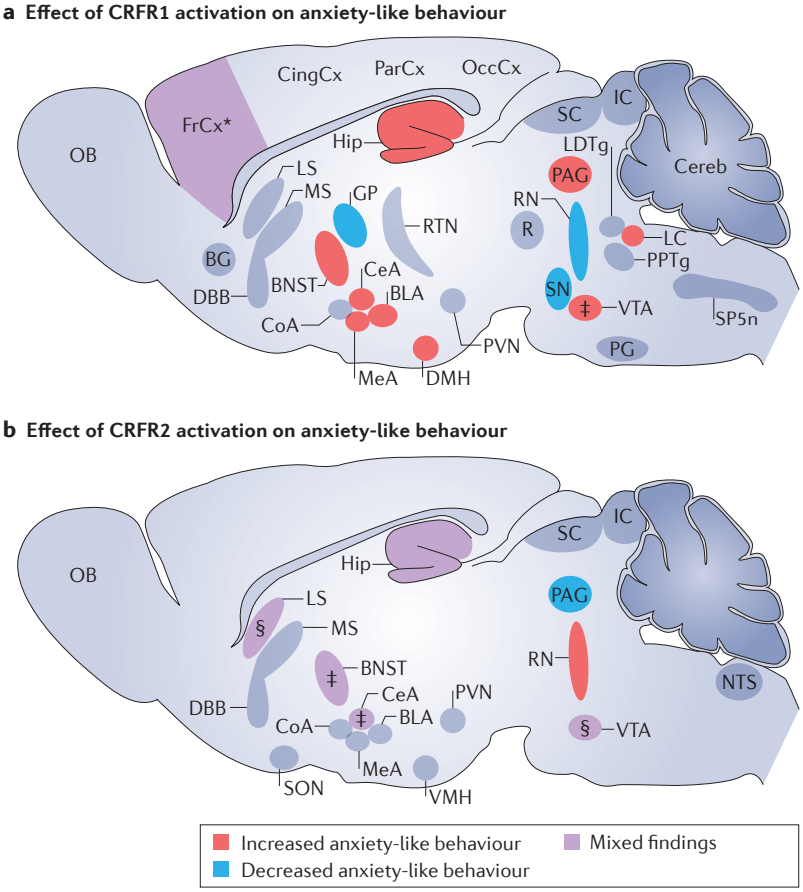

Figure 2 | **Region-specific effects of CRFRs on anxiety-like behaviour. a** | Effects of acute brain region-specific corticotropin-releasing factor receptor 1 (CRFR1) activation on anxiety-like behaviour are depicted in a sagittal section of the rodent brain (for brain regions not covered in the main text and for a more detailed overview of behavioural findings, see Supplementary information S1, S2 (box, table)). Regions associated with increased anxiety-like behaviour are shown in red; regions associated with reduced anxiety-like behaviour are shown in blue; regions associated with mixed findings are shown in purple. **b** | Effects of acute brain region-specific CRFR2 activation on anxiety-like behaviour are depicted in a sagittal section of the rodent brain (for brain regions not covered in the main text and for a more detailed overview of behavioural findings see Supplementary information S1, S3 (box, table)). BG, basal ganglia; BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CeA, central amygdala; Cereb, cerebellum; CingCx, cingulate cortex; CoA, cortical amygdala; DBB, diagonal band of Broca; DMH, dorsomedial hypothalamus; FrCx, frontal cortex; GP, globus pallidus; Hip, hippocampus; IC, inferior colliculus; LC, locus coeruleus; LDTg, laterodorsal tegmental nucleus; LS, lateral septum; MeA, medial amygdala; MS, medial septum; NTS, nucleus of the solitary tract; OB, olfactory bulb; OccCx, occipital cortex; PAG, periaqueductal grey; ParCx, parietal cortex; PG, pontine grey; PPTg, pedunculopontine tegmental nucleus; PVN, paraventricular nucleus of the hypothalamus; R, red nucleus; RN, raphe nucleus; RTN, reticular nucleus; SC, superior colliculus; SN, substantia nigra; SON, supraoptic nucleus; SP5n, spinal trigeminus nucleus; VMH, ventromedial hypothalamus; VTA, ventral tegmental area. *Effects of CRFR1 activation seem to be dose dependent. ‡Effects of receptor activation seem to be synapse specific. §Effects of receptor activation seem to depend on previous history of stress or drug abuse.

was shown to increase phosphorylation of cAMP-responsive element-binding protein (CREB)[86] and the expression of serum/glucocorticoid-regulated kinase 1 (SGK1), a serine/threonine protein kinase that is also upregulated in response to stress or glucocorticoids and helps to regulate synaptic plasticity and memory[87].

Ventral tegmental area. The VTA — which is the origin of dopaminergic cell bodies and widely implicated in the drug- and natural-reward circuitry of the brain — displays high levels of *Crfr1* mRNA. CRF-related signalling in the VTA has been extensively investigated for its role in stress-induced modulation of drug-dependent behaviours. An in-depth description of all the data gathered on this issue is beyond the scope of this Review and has been covered by several excellent reviews[88–90]. Briefly, CRF excites most neurons in the VTA[91], potentiates excitatory postsynaptic currents (EPSCs)[92], increases dopamine neuron firing[93] and dopamine release[94], and increases local glutamate release, an effect that is most pronounced in drug-experienced animals[95–97].

These CRF-induced (or stress-related) effects have been attributed to CRFR1 activation, which, through the activation of the phospholipase C (PLC)–protein kinase C (PKC) signalling pathway, enhances the hyperpolarization-activated current and increases the firing rate of VTA dopamine neurons[93]. Moreover, in drug-experienced animals, CRF acts through CRFR1–PKA pathways[97].

Behaviourally, infusion of CRF into the VTA of drug-experienced animals mimics the effects of stress, in that it promotes the reinstatement of cocaine seeking[98,99]. By contrast, blocking CRFR1 in these animals prevents the aversive motivational response to drug withdrawal[100], reduces stress-induced locomotor sensitization to drug challenge and prevents escalated drug self-administration[101], prevents escalated binge drinking[96,102], and reduces cue- and stress-induced reinstatement of drug seeking[103] — all effects that propose an anxiogenic role for CRFR1 signalling in the VTA. However, a recent study[104] indicated that infusion of CRF into the VTA reduces the motivation to work for a food reward and decreases the release of dopamine in the nucleus accumbens (NAc) upon reward delivery (but not during reward expectancy), seemingly at odds with the stress-induced reinstatement of drug-seeking behaviours.

The effects of VTA CRF on dopamine release seemed to be pathway specific, as local infusion of CRF decreased dopamine release after stimulation of the pedunculopontine tegmental nucleus but increased dopamine release after stimulation of the BNST. Moreover, conditional knockout of *Crfr1* in dopamine neurons in the VTA and substantia nigra of mice reduced overall dopamine release in the PFC following acute stress[40] (although striatal dopamine levels might have been increased by this manipulation, as CRF has been separately reported to inhibit dopamine release by dopaminergic cells in the substantia nigra, which project to the striatum[105]). Remarkably, however, *Crfr1* knockout specifically in dopaminergic neurons of the VTA and substantia nigra increased anxiety-like behaviour, suggesting an anxiolytic role for CRFR1 in this specific neuronal subpopulation[40].

Brainstem. Brainstem nuclei — which are known to be involved in arousal-related noradrenergic signalling (the LC), mood-modulating serotonergic signalling (the RN) and in pain-related signalling and mediation of defensive

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Box 3 | **Stressor-specific effects of CRFR activation**

Different types of stressors require different physiological stress responses to optimally cope with them. Not only the nature (for example, physical or psychological) but also the severity and duration of the stressor have a great influence on the required neuronal response[182]. As the corticotropin-releasing factor (CRF) system acts immediately, exerting its neuromodulatory effects on target neurons within seconds of its release[146], this Review focuses primarily on the acute effects of CRF receptor (CRFR) signalling, either in the presence or the absence of a background of (chronic) stress. However, there is an extensive body of literature indicating that, in the case of long-term exposure, CRF exerts effects that are fundamentally different from its acute actions[77], and these effects have been linked to a depressive rather than an anxious phenotype[213,214]. For example, in the hippocampus, prolonged periods of exposure to CRF and CRFR1 activation have been shown to impair (rather than boost) hippocampal function. The initially potentiating effects of CRF (*in vitro*) here reverse, such that, with longer exposure, CRF induces a decline in field excitatory postsynaptic potentials (fEPSPs), obliterates short- and long-term synaptic plasticity, and blocks activity-induced polymerization of spine actin. CRF destabilizes and thus depletes thin dendritic spines, through a small GTPase RhoA-dependent mechanism, resulting in a reduction of small, potentiation-ready excitatory synapses[215]. These effects have been shown to be mediated by local CRFR1-induced activation of NMDA receptors; these receptors then recruit the calcium-dependent enzyme calpain, which triggers the breakdown of spine actin-interacting proteins[216].

In terms of behaviour, prolonged CRFR1 activation as a consequence of chronic stress has been associated with impaired hippocampal function. The typically detrimental effects of chronic stress on learning and memory are absent in mice lacking *Crfr1* in forebrain neurons[217], and the administration of a CRFR1 antagonist in wild-type mice immediately after exposure to a stressor rescued long-term potentiation (LTP) and restored the integrity of the dendritic structure[218]. Thus, in contrast to the potentiating effects of short-term exposure to physiological levels of CRF, exposure to the levels of the peptide that are presumed to be present with severe stress results in spine retraction and loss of synapses over more-protracted time frames[78]. In line with this, individuals with post-traumatic stress disorder (PTSD) often display memory impairments[219]; and the repeated administration of a selective CRFR1 antagonist prevented such cognitive impairment and the associated decrease in hippocampal neuronal excitability in a mouse model of trauma (or PTSD)[220]. These contrasting observations for short- and long-term exposure to CRF emphasize the importance of thoroughly investigating the effects of CRFR signalling over an extended timescale (that is, hours to days) and the importance of the mechanisms behind their persistence over time.

Last, the effects of a stressor (and associated CRFR activation) depend on the individual. Factors such as age, sex and genetics — often in interaction with life events — are known to be major determinants of the effects observed following activation of the CRF–UCN system[210,221].

behaviour (the PAG) — also display high levels of *Crfr1* mRNA (although *Crfr1* mRNA expression in the LC might be rather low in mice, as it is not consistently detected)[30,33].

The LC is the primary source of noradrenaline in the brain[106] and is thus important in mediating stress-induced arousal[107]. The tonic neuronal discharge rate of the LC is positively correlated with arousal state[108] and is phasically activated by salient stimuli in order to orient attention towards the stimulus[109]. Local injection of CRF was shown to increase activity (assessed by immediate-early gene expression) not only in LC neurons but also in LC projection regions, including the ventral lateral septum (ventral LS), BNST and CeA[110]. CRF shifts the mode of LC discharge from a phasic state to a high-tonic state by acting on local CRFRs[111]. In addition to increasing arousal, this facilitates the disengagement from ongoing behaviour and promotes behavioural flexibility in response to challenging environmental conditions — an important cognitive adaptation of the stress response[112,113]. In line with this, local injection of CRF suppressed sensory-evoked responses of thalamic and cortical neurons[114] and facilitated shifting of attention in a dynamic environment[115]. Although it is unclear whether the LC expresses only CRFR1 (REF. 116), the effects of CRF on LC neurons were prevented (or greatly attenuated) by a selective CRFR1 antagonist[117,118]. A recent study using optogenetic stimulation of projections of CRF-expressing CeA neurons to the LC indicated that these neurons are a primary source of activation for LC CRFR1 and thus greatly influence CRFR1-mediated modulation of anxiety-like behaviour[119].

In the LC, CRFR1 has been shown to be internalized after CRF administration, (repeated) stress exposure or chronic ethanol intake[34,116,120], reducing cellular sensitivity to CRF. Studies have implicated this LC CRFR1 downregulation in the adaptive coping response to stress, as rats that are resilient in the learned helplessness model of depression displayed reduced CRFR1 levels in both the amygdala and LC[121]. However, other studies have shown a stress-induced sensitization of LC firing to low doses of CRF[122,123], which would allow for an arousal response to mildly stressful situations that would typically have no effect in unstressed animals. Therefore, the modulatory actions of prior stress exposure seem to depend on the duration and severity of the stressor. In addition, sex seems to be a crucial factor here, as the rate of internalization of CRFR1 in the LC is lower in female rats, making them more sensitive to low levels of CRF and less adaptable to the additional recruitment of the receptor to the plasma membrane as a consequence of stress or drug dependence[124,125].

In the RN — the source of serotonergic neurons that regulate positive affect and are involved in depression — CRFR1 is expressed both in serotonergic and non-serotonergic (mainly GABAergic) neurons[126,127]. Activation of the receptor (with low doses of CRF) in this region has been associated with local inhibition of neuronal firing[128] and reduced release of serotonin in the NAc[129], lateral striatum[130] and LS[131], a region in which local serotonin signalling has been associated with increased anxiety-like behaviour[132]. The RN CRFR1-mediated reduction in serotonin release in these regions has been suggested to allow for more-proactive (instead of typical anxious) behaviour, such as exploration, under mildly stressful conditions[133]. However, another study showed that repeated CRF-mediated CRFR1 activation in the dorsal RN had anxiogenic effects, contributing to anxiety induced by drug withdrawal[76], whereas selective CRFR1 disruption in RN serotonergic neurons was recently shown to have no effect on anxiety-like behaviour[40]. As CRF-containing axonal fibres topographically innervate the RN, it has been suggested that CRF may specifically alter the activity of distinct subsets of serotonergic (and GABAergic) neurons in this area[133]. This regional specificity of CRFR1 activation within the RN itself needs to be further investigated.

In the PAG, CRF injection excites neurons[134] and increases anxiety[135,136]. These anxiogenic effects have been related to CRFR1 activation; infusion of a CRFR1

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Table 2 | **Neuronal effects of regional manipulation of CRFR2-mediated signalling**

Brain region	CRFR2 manipulation*	Neuronal effects	Role for CRFR2 signalling	Refs
Central amygdala	Activation, low stress	Increased mEPSC amplitude and frequency	Excitation	58
		Increased sEPSC frequency	Excitation	55
		Enhanced evoked EPSCs	Excitation	58
	Inhibition, low stress	Depressed evoked EPSCs	Excitation	58
		No effect on evoked EPSCs	-	57
		Increased evoked spiking frequency	Inhibition	139
		No effect on evoked spiking frequency	-	139
	Inhibition, high stress	Increased mEPSC frequency, decreased PPR, enhanced evoked EPSCs	Inhibition	57
Lateral septum	Activation, low stress	Reduced mEPSCs (frequency and amplitude), depressed EPSC amplitude	Inhibition	58
	Activation, high stress	Enhanced evoked EPSCs, increased mEPSC frequency‡	Excitation	147
Ventral tegmental area	Activation, low stress	Potentiated NMDAR-mediated synaptic transmission	Excitation	151
		Reduced EPSCs, enhanced IPSCs	Inhibition	92
		Increased EPSCs, reduced IPSCs‡	Excitation	92
Raphe nuclei	Activation, low stress	Reduced neuronal discharge	Inhibition	153
	Activation, high stress	Increased neuronal discharge	Excitation	128,153
		Increased serotonin release in the nucleus accumbens	Excitation	129
		Blocked CRF-mediated increase in serotonin release in the nucleus accumbens	Excitation	129

CRFR2, corticotropin-releasing factor (CRF) receptor 2; EPSC, excitatory postsynaptic current; IPSC, inhibitory postsynaptic current; mEPSC, miniature EPSC; NMDAR, NMDA receptor; PPR, paired-pulse ratio; sEPSC, spontaneous EPSC. *Studies describing the effects of acute activation or inhibition of CRFR2 by a single administration of agonist or antagonist. ‡Effect of CRFR2 signalling after repeated administration of drugs of abuse.

agonist directly into the dorsal PAG increased avoidance behaviour[137], whereas administration of a CRFR1 antagonist blocked stress-induced anxiety-like behaviour[135], inhibited the dose-dependent anxiogenic and antinociceptive effects of CRF[138] and facilitated escape behaviour[136]. These results support a role for CRFR1 signalling in PAG excitation and increased defensive behaviours.

Region-specific functions of CRFR2

Compared with *Crfr1* mRNA, *Crfr2* mRNA in the rodent brain has a much more localized distribution pattern that is virtually confined to subcortical structures[32,33]. The highest levels of *Crfr2* expression are found in the LS, the ventromedial hypothalamic nucleus and the choroid plexus, whereas moderate levels are evident in the olfactory bulb, nuclei of the extended amygdala, hippocampus, PVN and supraoptic nuclei of the hypothalamus, inferior colliculus and RN[32,33] (FIG. 1b). However, it should be noted that these are sites of *Crfr2* mRNA expression and not of the protein, which may show a different distribution pattern.

Only limited data are available on brain region-specific modulation of CRFR2 signalling. Among the most investigated regions are the extended amygdala, hippocampus, LS, mPFC, VTA and brainstem nuclei (specifically, the RN and PAG). Below, we review the region-specific roles of local CRFR2 activation in the modulation of anxiety-like behaviour (FIG. 2b; TABLE 2).

Nuclei of the extended amygdala. Like *Crfr1*, *Crfr2* is expressed in the medial amygdala and BNST but, unlike *Crfr1*, it is only modestly expressed in the CeA and BLA[32,33]. In the BLA, although CRF-induced anxiogenic effects have mainly been attributed to CRFR1 signalling, one study claimed that microinjection of UCN has more potent effects than does microinjection of CRF[42]. Similar to CRFR1, mixed effects of CRFR2 activation in the CeA have been reported. In contrast to CRFR1 activation, though, at low stress levels CeA CRFR2 binding seems to facilitate synaptic transmission through both presynaptic and postsynaptic mechanisms[55,58]. However, other studies have shown that blocking CRFR2 signalling in the CeA increased evoked neuronal spiking frequency[139], spontaneous glutamate release and EPSCs in an arthritic pain model[57], leaving this issue open for further investigation.

Findings regarding CRFR2 signalling in the BNST are also complicated. Infusion of a CRFR2 antagonist into the anterolateral BNST increased anxiety and decreased the somatic mechanical threshold[75], whereas reduced expression of *Crfr2* mRNA in the posterior BNST has been related to a PTSD-like phenotype in rats that is attenuated by local *Crfr2* overexpression[140]. These data are consistent with an anxiolytic role for BNST CRFR2 signalling. However, other studies have reported a decrease in stress-induced startle after intra-BNST treatment with a CRFR2 antagonist[75], an aversion for the environment paired with BNST CRFR2 activation in a place-conditioning paradigm[73] and elevated

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levels of *Crfr2* in the BNST associated with a PTSD-like phenotype in mice that was rescued by BNST-specific *Crfr2* knockdown[141].

These apparent inconsistencies could potentially be explained by contrasting modulatory effects of CRF on the different subnuclei and cell types within the BNST. At least three cell types — differing in their patterns of intrinsic membrane currents — have been identified in the BNST[142]. Moreover, as the BNST integrates both topographically organized excitatory and inhibitory emotional and nociceptive signals from multiple brain regions[143,144], CRFR2 could both facilitate and attenuate output behaviour depending on its subregional localization. A similar explanation could possibly account for the contradicting findings on the CeA, where CRFR2 has been associated with both an increase in glutamatergic signalling[55,58] and a decrease in such signalling through the disinhibition of presynaptic GABA release[57]; these effects might thus depend not only on the brain region but also on the specific synapse that is targeted.

Lateral septum. The LS plays a part in reward processing and reinforcement and has been implicated in the modulation of fear and anxiety. GABAergic neurons in the LS express high levels of *Crfr2* mRNA[145].

Administration of UCN1 to the LS of rat-brain slices depressed excitatory glutamatergic transmission in this region, in both a phasic and a tonic mode, through presynaptic and postsynaptic mechanisms[58]. However, stress caused by chronic cocaine administration and subsequent withdrawal altered the sensitivity and function of local UCN signalling; the CRFR2-mediated depression induced by UCN1 was switched to a facilitation of glutamatergic transmission, with a comparable potency. This switch has been linked to a change from a PKA-dominant pathway to a PKC-dominant pathway activated downstream of CRFR2 (REFS 146,147).

These *in vitro* findings may support *in vivo* data that suggest altered behavioural effects of CRFR2 activation in the LS following stress. For example, local CRFR2 activation induced by UCN2 had hardly any effect under low-stress conditions, whereas it markedly increased anxiety under high-stress conditions[63]. Similarly, blockade of septal CRFR2 under basal conditions had no clear behavioural effects[28], whereas it reduced shock-induced freezing (that is, under high stress)[148,149]. Other studies confirmed these anxiogenic effects of local CRFR2 activation under high stress, showing that LS CRFR2 mediated the anxiety-inducing effects of immobilization stress, as well as the stress- or CRF-induced impairment of context-dependent fear conditioning[28,150]. Moreover, optogenetic manipulation of CRFR2-expressing neurons in the LS revealed that their activation is associated with acute anxiogenic effects (that is, a fear response) and a prolonged state of anxiety, whereas their inhibition induces an anxiolytic phenotype[145], indicating that activation of these neurons may indeed contribute to anxiety.

Ventral tegmental area. In the VTA, CRFR2 activation was shown to induce the potentiation of NMDA receptor-mediated synaptic transmission in dopamine neurons through activation of the PLC–PKC pathway[151]. CRFR2 signalling in the VTA has been reported to mediate the stress-induced reinstatement of drug-seeking behaviour, as well as the associated increases in local glutamate and dopamine release, as these stress effects were prevented by local CRFR2 blockade and mimicked by local administration of a CRFR2 agonist[98]. Moreover, CRFR2 was shown to be involved in stress-induced dopaminergic sensitization (that is, an increase in dopamine release in the NAc) and in the escalation of drug self-administration during a 'binge' (REF. 101); these data, together with the findings regarding the actions of CRFR1 on these processes, suggest complementary roles for CRFR1 and CRFR2 activation in the VTA.

However, findings of a recent study indicate that these effects may again be dependent on the history of stress or drug exposure of the animal[92]. In naive animals, activation of presynaptic CRFR2 reduced EPSCs and enhanced inhibitory postsynaptic currents (IPSCs) through heterosynaptic facilitation of GABAergic synapses; however, these effects of CRFR2 activation were diminished after chronic self-administration of cocaine and were even reversed by administration of yohimbine (which increases noradrenaline levels by inhibiting α2-adrenergic receptors) plus cue reinstatement through a reduction of tonic GABA-dependent inhibition. Thus, presynaptic CRFR2 seems to tightly regulate glutamate transmission in (and activation of) the VTA in a concerted, heterosynaptic manner.

Brainstem. *Crfr2* mRNA and protein are also expressed in both serotonergic and non-serotonergic (primarily GABAergic) neurons in the RN[127,152]. In contrast with the effects of local CRFR1 activation, activation of RN CRFR2 by high doses of CRF increases RN neuronal discharge[128,129], whereas CRFR2 blockade has the opposite effect[129]. This effect seems to be dose dependent and cell-type specific, as relatively low doses of UCN2 were found to inhibit most serotonergic RN neurons, but higher doses inhibited the majority of non-serotonergic (primarily GABAergic) RN neurons, resulting in activation of serotonergic neurons through disinhibition[153]. Stress exposure has been shown to increase both the proportion of responsive serotonergic RN neurons and the magnitude of the excitatory response to CRF, which raises the possibility that CRF actions on mesolimbocortical serotonergic systems may contribute to the development and expression of stress-induced behavioural sensitization[154]. This increase in the responsiveness and firing rate of serotonergic cells in the RN might be established by the recruitment of CRFR2 residing in the cytoplasm to the plasma membrane and the internalization of CRFR1 in the RN[127], but the exact underlying mechanisms remain unknown.

Behaviourally, RN CRFR2-induced serotonin release in the striatum[155], BLA[156], mPFC[157] and LS[132] has been associated with an increase in anxiety and fear-like behaviours, and accompanies the phenotype observed after uncontrollable, inescapable stress (that is, impaired escape behaviour and potentiation of fear conditioning), with local UCN2 (or high levels of CRF) mimicking,

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and CRFR2 antagonism blocking, such behavioural change[158,159]. Thus, CRFR2 activation can either increase or decrease the tone of the RN serotonergic system in a dynamic manner, depending on prior experience and on the level of ligand that is released.

Relatively little is known about the effects of CRFR2 signalling in the PAG. Although the anxiogenic and antinociceptive effects induced by infusion of CRF into the PAG are not mediated by CRFR2[138], the activation of CRFR2 does seem to impair avoidance learning in the elevated T maze[136], indicating an anxiolytic effect of CRFR2 signalling in this region.

Mechanisms for region-specific effects

The data described above clearly indicate that CRFR1 and CRFR2 modulate anxiety-like behaviour in rodents in a brain region-dependent manner. One obvious explanation for the differential behavioural effects of CRFR activation is that distinct brain regions simply serve distinct behavioural functions, and thus their activation induces a distinct behavioural phenotype. Activation of brain regions that are implicated in emotional arousal, such as the extended amygdala, is expected to increase anxiety, whereas activation of cognitive-control regions, such as the mPFC, is expected to contribute to stress coping and reduce anxiety. However, the contrasting findings in terms of both behavioural and electrophysiological effects of CRFR subtype activation, as observed within single brain regions, implicate a more complex underlying mechanism of region dependency and suggest specificity at the circuit, cell-type and ligand levels that depends on prior experience.

Circuit specificity. It is important to note that, in contrast to classical neurotransmitters (such as glutamate and GABA), CRF and UCN ligands primarily act as neuroregulators, 'priming' the subsequent targets of a neurotransmitter or other neuromodulator without inducing any apparent change in the membrane potential or electrical excitability of the neuronal membrane itself[160]. Therefore, the actual source of CRF or UCN ligands (and the co-release of other neuromodulators by this source) seems to be particularly important in determining the effects of local CRFR activation. Ligands of the CRF–UCN family are co-expressed with different neurotransmitters throughout the brain. For example, *Crf* expression is found in glutamatergic neurons (for example, in the PVN[161] and CeA[162]), GABAergic neurons (in the BNST[161,163], CeA[162,163] and hippocampus[164]), dopaminergic neurons (in the VTA)[100] and cholinergic neurons (in the brainstem)[165]. The exact source of a CRFR ligand and its associated neurotransmitter may therefore be crucial in determining whether it has an excitatory or an inhibitory modulatory effect.

Nevertheless, most of the studies described in this Review — in which exogenous ligand was applied to artificially activate CRFRs — neglected the effects of natural co-release of other neuromodulators. In addition, the local CRFR blockade that was implemented in these studies does not accurately isolate the effects of endogenous ligand release, as it is unspecific with respect to the actual activated synaptic contacts. The fact that several brain regions have multiple sources of endogenous ligand — each with potentially differential effects, given that these sources probably are activated under different conditions, have a distinct neurochemical composition and make unique synaptic contacts at their target site — may even explain the contrasting findings observed for a single brain region. Therefore, future studies should target these circuit-specific effects of CRFR activation.

Similarly to these afferent-specific effects, the effects of local CRFR activation may depend on the efferents of the affected neuron. For example, CRFR2-expressing LS neurons make both local and long-range inhibitory synapses in the hypothalamus, medial amygdala, PAG and ventral hippocampus[145]. Currently, it is not clear whether these synapses originate from the same neurons or instead are formed by distinct neuronal subsets with differential functions, but one could appreciate that the activation of each of these synapses may result in distinct electrophysiological and behavioural effects. Some studies have targeted circuit-specific effects of activation of the CRF–UCN system at the afferent[119,162,166] and efferent[145] levels, but additional studies are necessary to further explore potential circuit-specific effects of CRFR activation on anxiety-like behaviour.

Cell-type specificity. CRFR1 and CRFR2 seem to modulate anxiety-like behaviour in a cell type-dependent manner. How could this cell-type dependency come about? First of all, the effects of CRFR1 and CRFR2 activation are hypothesized to depend on the neuronal cell type in which the receptors are expressed (FIG. 1b), because distinct cell types are differentially affected by receptor activation or serve different functions (such as inhibitory versus excitatory neurotransmission). For example, high levels of CRF activate serotonergic RN neurons (presumably through CRFR2) but suppress non-serotonergic RN cells[153], and CRF injection into the BLA increases FOS expression in pyramidal cells but reduces GABA levels and GABAergic interneuronal activity[38]. Conditional inactivation of *Crfr1* in specific cell types has offered valuable insights into the possible functions of the receptor in these different neurotransmitter circuits. Conditional inactivation of *Crfr1* in GABAergic or serotonergic neurons had no determined effects on anxiety, indicating that the interaction between the CRF and serotonergic systems is not directly mediated by CRFR1 on serotonergic neurons but rather takes place at the postsynaptic level of the target or in input neurons in which CRFR1 and serotonin receptors are co-expressed[24,167]. Interestingly, mice lacking CRFR1 in glutamatergic circuits show reduced anxiety and impaired neurotransmission in the BLA and hippocampus, whereas mice lacking CRFR1 in dopaminergic neurons show increased anxiety-like behaviour and reduced dopamine release in the PFC[40]. This observed dual role for CRFR1 was suggested to indicate that, under physiological conditions, CRFR1-controlled glutamatergic and dopaminergic systems might function in a concerted but antagonistic manner to keep adaptive anxiety responses to stressful situations in balance[40]. This would imply that the CRF-signalling hyperactivity that is observed in many patients

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suffering from emotional disorders[168], such as major depressive disorder[11] and PTSD[12], might not be general but instead restricted to particular neuronal circuits. Such a dysregulation of CRF signalling might contribute to symptoms of such disorders by generating an imbalance between CRFR1-controlled glutamatergic and dopaminergic neuronal circuits involved in emotional behaviour. Similarly, CRFR2 is expressed in glutamatergic, GABAergic, serotonergic and dopaminergic neurons[151,152,169] (FIG. 1b), but no studies have yet systematically analysed its contribution to anxiety-like behaviour in these specific neuronal subsets. Thus, CRFRs might affect anxiety-like behaviour by modulating neuronal activity in a circuit-specific as well as a cell type-dependent manner.

Ligand and molecular pathway specificity. CRFR-mediated region-specific modulation of anxiety might be related to brain site-specific ligand binding and to the activated signalling pathways that are associated with it. As mentioned before, CRFR ligands display unique, partially overlapping expression patterns throughout the brain[170–173] (FIG. 1a). Although the sites of mRNA expression of these ligands are well established, it is largely unknown from which part of the neuron (that is, the axon, dendrite or synapse) the ligands are released. Further research on the exact (subcellular) release and target sites of these ligands is necessary to increase our understanding of their effects on CRFR signalling.

Although CRFRs primarily act through activation of the cAMP–PKA pathway (BOX 1), they have the ability to interact with other G protein systems as well, such as $G_q\alpha$, G_i, G_o, $G_{i1/2}$ and G_z (REF. 174), allowing for the modulation of a wide range of signalling pathways[175]. Moreover, particular conformations of ligand–receptor interaction seem to selectively induce different signalling cascades, further contributing to the brain region-specific actions of CRF and UCNs. For example, in CHO (Chinese hamster ovary) cells transfected with *Crfr2*, binding of UCN2 or UCN3, but not CRF, to CRFR2 selectively activates extracellular signal-regulated kinase–mitogen-activated protein kinase (ERK–MAPK) signalling[176]. Similarly, although the binding of UCN1 or CRF to CRFR1 in the hippocampus induces CREB phosphorylation, UCN1 binding, but not CRF binding, induces this process via a MEK–MAPK-dependent pathway[86].

Besides the activated pathway, desensitization and receptor internalization following receptor activation also seem to depend on the specific ligand that is bound. Desensitization of CRFR2–cAMP signalling was shown to occur more rapidly and to a greater extent in response to UCN2 than to UCN3, whereas CRF is a relatively weak desensitizing agonist[177]. Also, the internalization of CRFR2 is greater upon exposure to UCN2 than upon exposure to CRF[178]. Furthermore, differential neuronal expression of G protein-coupled receptor kinases (GRKs) and β-arrestins[179,180] (BOX 1), which are important regulators of CRFRs, may greatly influence regional regulation of CRFR1 and CRFR2 signalling.

Thus, the differential effects of distinct ligand binding, as well as of other modulators of the molecular signalling pathway activated by receptor binding, may also contribute to the brain-region specificity of the effects of CRFR activation. However, remarkably little is known about the exact signalling cascades that are downstream of CRFRs, particularly their specificity or their regulation. Further studies of these downstream molecular pathways are needed to assess the mechanistic underpinnings of the effects of local CRFR activation.

Conclusions and outlook

Above, we have reviewed data on brain region-specific modulation of anxiety-like behaviour via CRFRs that were acquired from studies manipulating the receptors site-specifically, through conditional mutagenesis, pharmacological intervention, viral manipulation or optogenetics. These findings reveal a more complicated modulatory role of the CRF–UCN system than the conventional view, in which CRFR1 induces anxiety and CRFR2 mediates stress recovery. Although in most brain regions covered in this Review (including the BLA, BNST and PAG) CRF-mediated activation of CRFR1 increases basal and stress-induced anxiety, a more complex picture is emerging for other brain regions. In the globus pallidus, for example, activation of CRFR1 is associated with anxiolytic effects, whereas the potential anxiogenic effects of CRFR1 activation in the CeA seem to depend on current stress levels and stress history[63,64,67]. A dependency on current and previous stress levels is also observed in the NAc. In this region, CRF-mediated activation of CRFR1 induces a positive affective state (associated with appetitive stimuli) and increases dopamine release in naive mice[181], but severe stress exposure completely and persistently abolishes this effect and switches the behavioural response to NAc CRF from appetitive to aversive. In addition, the effects of CRFR2 activation seem to be dynamic (that is, plastic) and heavily dependent on the level of ligand present and prior experience (that is, history of stress exposure or drug addiction) (TABLE 1). CRFR2 signalling may indeed contribute to a coping mechanism in response to stress, although it is too early to say whether these changes reflect adaptive or maladaptive coping processes.

Some limitations to the described work and methods have to be mentioned as well. Most studies on regional manipulation of the CRF–UCN system have relied on the administration of exogenous neuropeptides (agonists or antagonists). These studies therefore do not capture the complexity of the circumstances of endogenous ligand release, which involves a 'neurosymphony' of stress mediators — neurotransmitters, neuropeptides and steroids — that are released throughout the entire brain[182]. We are only starting to unravel the entanglement of these systems and the neural circuits and networks involved. Future studies should therefore not only investigate the isolated, region-dependent effects of CRFR activation and their underlying mechanisms but also their contribution to neural networks and their interaction with other systems. This will ultimately advance our understanding of the role of the CRF system in mediating anxiety and stress-related behaviour and improve the treatment options of stress-related mental disorders such as major depressive disorder and PTSD.

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Competing interests statement

The authors declare no competing interests.

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